Cumulative Examination in Biochemistry
Metabolism and Thermodynamics

1. Identify at least 5 different ways metabolic pathways can be regulated in living organisms. Illustrate your answer with specific examples from metabolism.

   Brief Ans. Any 5 of the following ways of multi-level metabolic regulation could be discussed and illustrated with specific examples from metabolism:
   
   - Control of enzyme activity
     - Reversible (non-covalent) inhibition:
       - Competitive inhibition
       - Non-competitive inhibition
       - Uncompetitive inhibition
       - Allosteric feedback (positive or negative) regulation via either of above 3
       - Feed-forward activation
       - Conformational change e.g. kinase/phosphatase activity
       - pH or redox potential changes
     - Irreversible (covalent) enzyme inhibition
   - Control of enzyme levels
     - Enzyme synthesis: regulation of genes, transcription, translation etc
     - Enzyme degradation (proteolysis)
     - Disease-related up- or down-regulation
   - Control by substrate concentration and availability
   - Control through substrate cycling (often hormone-related)
   - Control through demand for product
   - Control by energy (ATP) availability
   - Control through compartmentalization
   - Hormonal regulation, signal transduction and signal cascades
   - Nervous and neurotransmitter control

2. Discuss the thermodynamic characteristics of metabolic reactions that are primarily controlled by a) changes in substrate concentration and, b) enzyme inhibition. Illustrate your answer with specific examples from metabolism.

   Brief Ans.

   i) First introduce the concept of steady state and explain how near-equilibrium, far-equilibrium & flux-determining reactions regulate metabolism by maintaining steady state or changing flux from one steady state to another

   ii) Then introduce the appropriate thermodynamic formulae to explain the relationship between Gibbs' free energy function, the equilibrium constant and concentration. Then use the following formula to determine the equilibrium status of in vivo reactions- whether they are near- or far-equilibrium:

   \[ \Delta G_{eq} = -RT \ln K_{\text{eq}} / \Gamma \] where \( \Gamma = \text{the mass action ratio in vivo} \]
a) Metabolic reactions that are primarily controlled by changes in substrate concentration are near-equilibrium, highly reversible and substrate-limited reactions:

$$
\Delta G \approx 0, \frac{[P]}{[S]} \approx \frac{[P]}{[S]}, \Gamma \approx K, \text{ the ratio } K / \Gamma \approx 1
$$

Rates of forward and reverse reactions similar to near-equilibrium and

$$
[S]_{in \text{ vivo}} \ll K_m \text{ enzyme activity controlled by } [S] \text{ i.e. 1st order (i.e. substrate-limiting).}
$$

Such a reaction cannot maintain constant flux or a steady state situation.

Enzyme activity change will not necessarily affect the flux;

Reactions reversible; small changes in [S] and [P] produce large and rapid changes in flux

Reactions very sensitive to changes in flux and direction but not to enzyme regulation as enzyme concentration exceeds that of substrate concentration (enzyme is NOT limiting)

b) Metabolic reactions that are primarily controlled by enzyme inhibition are far-equilibrium, mainly irreversible and enzyme-limited reactions

$$
\Delta G \text{ large -ve; } \frac{[P]}{[S]} \ll \frac{[P]}{[S]}, K / \Gamma \gg 1
$$

Far-equilibrium tend to have low forward velocity and 1 000-fold lower (quantitatively insignificant) reverse velocity; reaction effectively irreversible.

Far-equilibrium reactions determine direction of metabolic pathways.

Far-equilibrium reactions of lowest net activity in each pathway make the major contribution to pathway flux; the more far-equilibrium a reaction the greater contribution to flux control.

Enzymes are saturated with substrate; $[S]_{in \text{ vivo}} > 10xK_m$; Decrease in [S] will not affect enzyme activity i.e. zero order; a constant flux (at $V_{max}$ if enzyme not inhibited) maintained through the whole pathway. Change in enzyme activity by e.g. enzyme inhibition, hormonal action, or allosteric or gene control, or pH or temperature changes etc, causes change in pathway flux.

**NB.** But both near- and far-equilibrium reactions contribute to pathway flux just to different extents.

3. Consider the supplied excerpts from two journal articles and address the following questions:

- **For the supplied 2006 paper:**

  a) Interpret all the data and draw clear conclusions from the findings;

  Thermodynamic analysis is only rarely applied in biotechnology in order to judge the potential of prospective microbial strains and systems with respect to their growth and bioproduct synthesis performance. Yet, the literature shows thermodynamics to hold considerable promise in this respect. As shown by several authors, the wide variations of biomass yields reported for different microbial growth system can be explained based on thermodynamic reasoning. These variations appear to be the result of an evolutionary adaptation of the amount of Gibbs energy dissipation towards a reasonable compromise between growth efficiency and growth rate.
Based on this framework, biomass yields may be predicted very roughly for oxidative and reductive chemoheteroorganotrophic growth but also for chemolithothrophic. This type of analysis has yet to be extended to phototrophic growth. Similar analyses have been developed for other important growth parameters including maintenance coefficients, specific growth rate and threshold substrate concentrations. The state of affairs of thermodynamics for assessing individual metabolic pathways is much worse. As a result, thermodynamic analysis might be most useful in environmental applications, but is essentially unable at the time being to predict biomass yields in cultures with highly complex nutritional patterns such as mammalian cell cultures or yields of products not related to the energy metabolism such as overproduced primary and secondary metabolites, or recombinant proteins. Much more work has to be invested in the thermodynamics of processes in the living cells. Most important, our database concerning the Gibbs energy of the chemicals of life and the biochemical reactions, but also our knowledge on intracellular chemicals affecting the forces driving these reactions must be dramatically improved. This includes more accurate and more detailed data on the currency metabolites, whose concentrations have a decisive impact on thermodynamic calculations. In conclusion, more research should be aimed at a quantitative description of the intracellular environment in which life processes occur.

b) Write a well-structured abstract for this paper;

**Actual Abstract in Paper (see below):** I would expect a better structured abstract than this as follows: Background/context, motivation for study (what has/hasn’t been studied- deficiencies in knowledge), research questions, methods used to address questions, major findings (how the contribute to new knowledge), implications for future research

This paper attempts to review in how far thermodynamic analysis can be used to understand and predict the performance of microorganisms with respect to growth and bioproduct synthesis. In the first part, a simple thermodynamic model of microbial growth is developed which explains the relationship between the driving force for growth in terms of Gibbs energy dissipation and biomass yield. From the currently available literature, it appears that the Gibbs energy dissipation per C-mol of biomass grown, which represents the driving force for chemotrophic growth, may have been adapted by evolutionary processes to strike a reasonable compromise between metabolic rate and growth efficiency. Based on empirical correlations of the C-molar Gibbs energy dissipation, the wide variety of biomass yields observed in nature can be explained and roughly predicted. This type of analysis may be highly useful in environmental applications, where such wide variations occur. It is, however, not able to predict biomass yields in very complex systems such as mammalian cells, nor is it able to predict or to assess bioproduct or recombinant protein yields. For this purpose, a much more sophisticated treatment that accounts for individual metabolic pathways separately is required. Based on glycolysis as a test example, it is shown in the last part that simple thermodynamic analysis leads to erroneous conclusions even in well-known, simple cases. Potential sources for errors have been analyzed and can be used to identify the most important needs for future research.

c) Select any ONE figure and illustrate an alternative way of visualizing/processing the data;

Figures 1-3 could simply be re-drawn to depict an alternate way of represent the information portrayed. 
Figure 4-11 could be re-drawn to depict any alternate way of representing the same data.
Brief Answer Key

d) Critically compare and contrast the way you suggest in "c" with the way it is done in the paper in terms of how well the data processing supports the interpretation reached from the findings:

- For Table S1 below:

e) Use the pH data in the Table S1, as well as the findings in the 2006 paper, to explain why it is important to consider compartmentalization when studying metabolism and its thermodynamics.

Major points to make:
- Each compartment has enzymes catalyzing key reactions.
- Each enzyme and protein modulator (e.g. transcription factor) will have a unique pH optimum which keeps it active (give example of bell-shaped curve for effect of pH on enzyme activity)
- In addition, reaction mechanisms such as acid-base catalysis and any reaction that has hydrogen or hydroxyl ions in its equation require specific pH environments to occur
- Thus, most cellular reactions need specific pH environments to function and would not occur if in a different compartment; if hydrogen or hydroxyl ions participate in a metabolic reaction then the AG of the reaction will change
- Explain how pH changes can change the activity of an enzyme by titrating acidic and basic amino acid side chains, thereby changing its conformation or substrate charge interaction at the active site etc
- Some compartments such as lysosomes manipulate the pH via proton pumps to control enzyme enzymes
- In some compartments a particular pH keeps an enzyme inactive (latent) till needed to perform a particular function
- Membrane divided compartments allow for control of metabolic pathways
- Compartments also "use" changes in redox potential to control enzyme activity and therefore metabolism

Table S1: pH in each of eight cellular compartments

<table>
<thead>
<tr>
<th>Compartment</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol and nucleus</td>
<td>7.20</td>
</tr>
<tr>
<td>Extracellular fluid</td>
<td>7.40</td>
</tr>
<tr>
<td>Golgi apparatus</td>
<td>6.35</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>5.50</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>8.00</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>7.20</td>
</tr>
<tr>
<td>Peroxisomes*</td>
<td>7.00 ± 1.2</td>
</tr>
</tbody>
</table>

Inorganic Key - 3-2-13

0. \[ \sigma^2 \sigma^2 3\sigma^2 | \pi^4 2\pi^2 \quad \sigma^2 \sigma^2 3\sigma^2 | \pi^4 2\pi^1 \]

\text{triplet} \ (O_2) \quad \text{doublet} \ (NO)

2. \[ -\frac{d[\text{NO}]}{dt} = 2 \frac{d[\text{NO}_2^-]}{dt} = k \frac{[\text{NO}]^2 [O_2]}{} \]

(a).

\[ k \ (23^\circ C) = 8.4 \times 10^6 \text{ M}^{-2} \text{s}^{-1} \]

\[ k \ (37^\circ C) = 9.6 \times 10^6 \text{ M}^{-2} \text{s}^{-1} \]

(b). See table 1 or 1/2 if you remembered 2:1 stoichiometry \ ((NO) : \text{NO}_2^-) \]

(C). \text{Limiting O}_2 \gg \text{excess NO}

\text{first-order} \quad k_4 = k \frac{[\text{NO}]^2}{[O_2]}

\[ -\frac{d[O_2]}{dt} = k_4 [O_2] \]

\[ -\int \frac{d[O_2]}{[O_2]} = k_4 \int dt \]

\[ [O_2]_t = [O_2]_0 e^{-k_4 t} \]

\text{Limiting NO} \gg \text{excess O}_2

\text{second-order} \quad k_t = k \frac{[O_2]}{} \]

\[ -\frac{d[NO]}{dt} = k_t [NO]^2 \]

\[ -\int \frac{d[NO]}{[NO]^2} = k_t \int dt \]

\[ \frac{1}{[NO]_t} = \frac{1}{[NO]_0} + k_t t \]

(d). Two equations \& two unknowns

\[ -\frac{E_a}{k_B} = -0.00114 \]

\[ E_a = 1.57 \times 10^{-26} \text{ J molecule}^{-1} \]

Chemists prefer \[ \frac{-E_a}{R} \]

\[ E_a = 0.00947 \text{ J mol}^{-1} \text{ (still very small number)} \]

This reaction is governed by \( \Delta S^\circ \).

(E). \( \text{NO} + O_2 \rightleftharpoons \text{I} \)

\( \text{I} + \text{NO} \rightarrow 2\text{NO}_2 \)

\( \text{NO}_2 + \text{NO} + \text{H}_2\text{O} \rightarrow 2\text{NO}_2^- + 2\text{H}^+ \quad \text{first} \)

\( \text{NO}_2 + \text{NO} + \text{H}_2\text{O} \rightarrow 2\text{NO}_2^- + 2\text{H}^+ \quad \text{fast} \)
Problem 1. (20 pts)

Consider a hypothetical situation in which the pure rotational selection rule is:

\[ \Delta J = \pm 2 \text{ or } \pm 1 \]

instead of just \( \Delta J = \pm 1 \).

a. Obtain expressions for the energies at which allowed rotational transitions of a diatomic rigid rotor will be observed for this selection rule.

Rotational energies are at \( E_J = J(J+1)Bh \)

for \( \Delta J = \pm 2 \) the transition energies are:

\[ E_{J+2} - E_J = (J+2)(J+3)Bh - J(J+1)Bh = (4J+6)Bh \]

for \( \Delta J = \pm 1 \), transition energies are:

\[ E_{J+1} - E_J = (2J+2)Bh \]

The following transitions can occur:

- \( J = 0 \rightarrow J = 2 \) \( \Delta E = 6Bh \) 4Bh
- \( J = 1 \rightarrow J = 3 \) 14Bh
- \( J = 2 \rightarrow J = 4 \) 18Bh
- \( J = 3 \rightarrow J = 5 \) \( \Delta E = 2Bh \)
- \( J = 0 \rightarrow J = 1 \)
- \( J = 1 \rightarrow J = 2 \) 4Bh
- \( J = 2 \rightarrow J = 3 \) 6Bh
- \( J = 3 \rightarrow J = 4 \) 8Bh
- \( J = 4 \rightarrow J = 5 \) 10Bh

Combined, all transitions occur with spacing 2Bh, with the \( \Delta J = \pm 2 \) transitions overlapping every other line of the \( \Delta J = \pm 1 \) transitions.
b. For the case of a diatomic molecule with the preceding selection rule for which 2Bh corresponds to a wave number of 20.7 cm\(^{-1}\), calculate the expected intensity ratio of the absorption band at 10Bh to that at 6Bh at a temperature of 400 K. (You may assume that the absorption intensity is determined solely by the population of the initial state.)

The absorption band at 6Bh is from two transitions:

\[ J=0 \rightarrow J=2 \quad \text{and} \quad J=2 \rightarrow J=3 \]

The intensity of this band will therefore be proportional to the sum of the populations of the J=0 and J=2 states.

For the absorption band at 10Bh, we have a similar situation, but with the populations at J=1 and J=4.

Population in the \( j \)th state = \( N_j = C g_j e^{-\frac{(E_j)}{kT}} \)

where \( g_j = 2J+1 \) is the standard rotational degeneracy.

Therefore, intensity of band at 6Bh \( \propto N_0 + N_2 \)
and the intensity of band at 10Bh \( \propto N_1 + N_4 \)

\[
\frac{I(10\text{Bh})}{I(6\text{Bh})} = \frac{N_1 + N_4}{N_0 + N_2} = \frac{g_1 e^{-\frac{E_1}{kT}} + g_4 e^{-\frac{E_4}{kT}}}{g_0 e^{-\frac{E_0}{kT}} + g_2 e^{-\frac{E_2}{kT}}}
\]

\[
= \frac{3 e^{-\frac{2\text{Bh}}{kT}} + 9 e^{-\frac{10\text{Bh}}{kT}}}{1 + 5 e^{-\frac{6\text{Bh}}{kT}}}
\]

\[ \text{Bh}/kT \text{ for } T=400K = \frac{(10.35 \text{ cm}^{-1}) (1.986 \times 10^{-23} \text{ J/cm}^{-1})}{(1.381 \times 10^{-23} \text{ J/K}) (400K)} = 3.72 \times 10^{-2} \]

Therefore:

\[
\frac{I(10\text{Bh})}{I(6\text{Bh})} = \frac{2.785 + 4.275}{1 + 3.999} = \boxed{1.4}
\]
Problem 2. (30 pts)

Assume you have a system with evenly-spaced, singly-degenerate energy levels. Call the energy spacing $\epsilon$, and let the ground state have energy $\epsilon_0 = 0$.

a. What is the partition function, $Z$, for this system if it has an infinite series of energy levels?

$$Z = \frac{1}{1 - e^{-\beta \epsilon}}$$

b. What is the expression for the probability, $p_i$, of finding a particle in some level, $i$, for the system described above. (you can just call the partition function $Z$ and keep it as a variable).

$$p_i = \frac{e^{-\epsilon_i / kT}}{Z}$$

c. Now assume you have only three levels in your system. What are the expressions for the probabilities for each of these three levels, assuming $\epsilon_0 = 0$, $\epsilon_1 = \epsilon$ and $\epsilon_2 = 2\epsilon$.

The function given in part a comes from the series:

$$Z = \sum_{i=0}^{\infty} e^{-\beta \epsilon_i}$$

Therefore, for the system in this part of the problem:

$$Z = e^{-\beta \epsilon_0} + e^{-\beta \epsilon_1} + e^{-2 \beta \epsilon_2}$$

$$p_i = \frac{e^{-\epsilon_i / kT}}{Z}$$

So:

$$P_0 = \frac{1}{2}, P_1 = \frac{e^{\epsilon / kT}}{Z}, P_2 = \frac{e^{-2\epsilon / kT}}{Z}$$

d. At $T = 0$ K, what are the population distributions in these three levels? (i.e. what are the values of $p_0$, $p_1$ and $p_2$?)

$$P_0 = 1, P_1 = P_2 = 0$$

population is entirely in the ground state

e. As $T$ goes infinitely high, what are the population distributions in these three levels? (i.e. what are the values of $p_0$, $p_1$ and $p_2$?)

$$P_0 = P_1 = P_2 = \frac{1}{3}$$

for very high $T$. 
Problem 3. (25 pts)

The normalized wavefunction for a particle that is confined to go around in a ring (a circle in one plane), is given by:

\[ \psi(\phi) = \sqrt{\frac{1}{2\pi}} e^{-ik\phi} \quad \text{where } k = 0, \pm 1, \pm 2, \ldots \quad \text{and } 0 \leq \phi \leq 2\pi. \]

a. Find \( \langle \phi \rangle \).

\[ \psi^* = \sqrt{\frac{1}{2\pi}} e^{ik\phi} \]

\[ \langle \phi \rangle = \int_0^{2\pi} \psi^* \psi \, d\phi \]

\[ = \frac{1}{2\pi} \int_0^{2\pi} e^{ik\phi}(\phi)e^{-ik\phi} \, d\phi \]

Since \( e^{ik\phi} - e^{-ik\phi} = 1 \),

\[ \langle \phi \rangle = \frac{1}{2\pi} \int_0^{2\pi} \phi \, d\phi \]

\[ = \frac{1}{2\pi} \left[ \frac{1}{2} \phi^2 \right]_0^{2\pi} \]

\[ = \frac{1}{2\pi} \left( 2\pi \right)^2 \]

\[ \langle \phi \rangle = \pi \]
b. What is the kinetic energy of the particle? You can leave your answer in terms of $\hbar$, $k$, and $m$. (Note: the dimension of motion of this particle is $\phi$, not $x$.)

\[
\text{kinetic energy operator} = -\frac{\hbar^2}{2m} \frac{d^2}{d\phi^2}
\]

\[
\frac{d}{d\phi} e^{-ik\phi} = -ik e^{ik\phi}
\]

\[-ik \left( \frac{d}{d\phi} e^{ik\phi} \right) = -k^2 e^{ik\phi}
\]

Therefore:

\[
KE = \frac{k^2 \hbar^2}{2m}
\]
**Problem 4. (25 pts)**

The ro-vibrational spectrum (coupled rotation and vibration) of the molecule HI exhibits bands (P and R lines) at the following wavenumbers:

\[
\begin{align*}
P & \quad 2257.1 \text{ cm}^{-1} > 13.1 \text{ cm}^{-1} \\
   & \quad 2270.2 \text{ cm}^{-1} > 13.1 \text{ cm}^{-1} \\
   & \quad 2283.3 \text{ cm}^{-1} > 13.1 \text{ cm}^{-1} \\
   & \quad 2296.4 \text{ cm}^{-1} > 13.1 \text{ cm}^{-1} \\
R & \quad 2322.6 \text{ cm}^{-1} > 13.1 \text{ cm}^{-1} \\
   & \quad 2335.7 \text{ cm}^{-1} > 13.1 \text{ cm}^{-1} \\
   & \quad 2348.8 \text{ cm}^{-1} > 13.1 \text{ cm}^{-1}
\end{align*}
\]

Assume the ro-vibrational spectrum can be described by the equation:

\[E_{vJ} = (v + \frac{1}{2}) \hbar \nu_0 + J(J + 1) \hbar.\]

\[\text{a. Which of these lines are P-branch lines, and which ones are R-branch lines?}
\]

P-branch lines are at lower energies. Also, the gap that is twice as big as the other gaps is the space between the P and R branches (where the Q branch would be, if there were one.)

P lines: 2257.1, 2270.2, 2283.3, 2296.4

R lines: 2322.6, 2335.7, 2348.8

\[\text{b. What is the fundamental vibrational frequency of HI, expressed in units of cm}^{-1}?\]

It would be at the point right between the P and R branches:

\[
\nu_0 = \frac{(2322.6 - 2296.4)}{2} + 2296.4 \text{ cm}^{-1}
\]

\[= 2309.5 \text{ cm}^{-1}\]
c. Compute the equilibrium H-I bond distance using the given data.

Rotational bond spacings in the Por R branch are $2Bh$. So $2Bh = (13.1 \text{ cm}^{-1}) (hc)$

$$B = \frac{\hbar}{8\pi^2 I}$$

$$2Bh = \frac{\hbar^2}{4\pi^2 I} = (13.1 \text{ cm}^{-1}) (hc)$$

$$= 2.6022 \times 10^{-22} \text{ J}$$

Solving for $I$ gives:

$$I = \frac{\hbar^2}{4\pi^2 (2.6022 \times 10^{-22} \text{ J})}$$

$$= 4.2738 \times 10^{-47} \text{ kg m}^2$$

$$I = \mu R_e^2$$

$$R_e = \sqrt{\frac{I}{\mu}} = \sqrt{\frac{4.2738 \times 10^{-47} \text{ kg m}^2}{1.601 \times 10^{-27} \text{ kg}}}$$

$$R_e = 1.604 \times 10^{-10} \text{ m or 0.16 nm}$$
Potentially Useful Information

Atomic masses: $H = 1.0079 \text{ g/mol}$; $I = 126.9045 \text{ g/mol}$
speed of light: $c = 2.99792 \times 10^8 \text{ m/s}$
Planck's constant: $h = 6.626076 \times 10^{-34} \text{ J s}$

Avogadro's number: $N_A = 6.02214 \times 10^{23} \text{ mol}^{-1}$
Bolzmann constant: $k_B = 1.38066 \times 10^{-23} \text{ J K}^{-1}$
gas constant: $R = (k_B)(N_A) = 8.3145 \text{ J mol}^{-1} \text{ K}^{-1}$

$1 \text{ cm}^{-1} = 1.986 \times 10^{-23} \text{ J}$